molecular dynamics simulations to explore the role of the three loop regions in sustaining the bound complex

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Membrane-Mediated Induction and Sorting of K-Ras Microdomain Signaling Platforms
Katrin Weise, Shobhna Kapoor, Christian Denter, Jörg Nikolaus, Norbert Opitz, Sebastian Koch, Gemma Triola, Andreas Herrmann, Herbert Waldmann, Roland Winter.
The K-Ras4B GTPase is a major oncoprotein whose signaling activity depends on its correct localization to negatively charged subcellular membranes and nanoclustering in membrane microdomains. Selective localization and clustering are mediated by the polybasic farnesylated C-terminus of K-Ras4B, but the mechanisms and molecular determinants involved are largely unknown. In a combined chemical biological and biophysical approach we investigated the partitioning of semisynthetic fully functional lipidated K-Ras4B proteins [1] into heterogeneous anionic model membranes and membranes composed of viral lipid extracts. Independent of GDP/GTP-loading, K-Ras4B is preferentially localized in liquid-disordered (ld) lipid domains and recruits anionic lipids by an effective, electrostatic lipid sorting mechanism to form new protein-containing fluid domains with higher anionic charge density. In addition, GDP-GTP exchange and, thereby, Ras activation results in a higher concentration of activated K-Ras4B in the nanoscale signaling platforms. Conversely, palmitoylated and farnesylated N-Ras proteins partition into the ld phase and concentrate at the ld/lo phase boundary of heterogeneous membranes [2,3]. Next to the lipid anchor system, the results reveal an involvement of the G-domain in the membrane interaction process by determining minor but yet significant structural reorientations of the GDP/GTP-K-Ras4B proteins at lipid interfaces. A molecular mechanism for isoform-specific Ras signaling from separate membrane microdomains is postulated from the results of this study.
1.) Chen Y-X et al. (2010) Angew. Chem. Int. Ed. 49:6090–6095.
2.) Weise K et al. (2009) J. Am. Chem. Soc. 131:1557–1564.
3.) Vogel A et al. (2009) Angew. Chem. Int. Ed. 48:8784–8787.

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Elucidation of the Integrin Inside-Out Activation Mechanism
Antreas Kalli, Iain D. Campbell, Mark S.P. Sansom.
Integrins are major cell surface receptors that are crucial for a variety of cell migration and adhesion events. They are 'activated' to a high affinity state by the intracellular protein talin, a process known as ''inside-out activation''. In this process, complex formation between the talin head domain and the integrin β cytoplasmic tail, as well as talin/membrane interactions, are believed to play a crucial role. In this study, long multi-scale molecular dynamic simulations were used to probe the talin F2-F3/membrane and talin F3/β1D interactions in a POPC/POPG bilayer. A reorientation of the talin F2-F3 domain to optimize contacts with the negatively charged moieties in the membrane, followed by a large increase in the tilt angle of the β1D tail relative to the bilayer normal was observed. In addition, our simulations demonstrate that mutation of four basic residues in the F2 domain of talin, previously suggested to be involved in membrane interactions, reduces the affinity of talin F2-F3 for the membrane and changes its orientation relative to the bilayer surface. This perturbed orientation of talin relative to the membrane in the F2 mutant is expected, in turn, to perturb talin/integrin interactions. During the simulations, enrichment of the F2-F3 binding surface with anionic lipids reveals an important role for negatively charged moieties in the membrane. On the basis of these simulations, a model for disruption of the integrin α/β transmembrane (TM) interactions is proposed in which the large increase in the tilt angle to the β tail upon talin binding weakens the α/β TM association, destabilizes the α/β dimer thus leading to integrin tail separation.

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The Number of Lipid Droplets in the Fission Yeast S. pombe is a Function of the Cell Cycle Stage
Allan Long, Anna Manneschmidt, Rose Dortch, Robert Verbruggie, **Paul Dalhaimer**.
Lipid droplets are cellular storage centers for neutral lipids. They are composed of a phospholipid monolayer with bound proteins surrounding a core of neutral lipids. They are typically viewed as relatively long-term storage depots for excess neutral lipids. We use fluorescent microscopy to show that lipid droplets are highly dynamic entities in terms of number and size in the fission yeast *Schizosaccharomyces pombe* when grown in media lacking excessive amounts of fatty acids. The average number of BODIPY-strained lipid droplets in fission yeast cells, *n*, changes during the cell cycle: S (*n*) → G2 + M (~2*n*) → G1 (*n*) as measured by epi fluorescence. The value for *n* is typically ~10. We used confocal microscopy to monitor the presence of the fluorescent lipid droplets in each slice of the acquired stack. In this way, we measured the change in size of the lipid droplets over time. Where most lipid droplets showed fluctuations in size of tens of nanometers or less per minute, a subset of the lipid droplets expanded and shrank at rates of up to 500 nm/min. The formation and complete lipolysis of lipid droplets is directed: the droplets do not form and disappear around the center slice. The spatially directed reduction in the size of LDs seems to strengthen the hypothesis that LDs are lipolysized by other organelles and not by freely diffusing enzymes.

Synaptic Transmission

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Protein Oxidation Inhibits Nitric Oxide-Mediated Signaling Pathway Essential for Synaptic Plasticity
Sho Kakizawa, Masahiko Shibazaki, Nozomu Mori, Hiroshi Takeshima.
Oxidative stress is a primary factor inducing brain dysfunction in aged animals. However, how oxidation affects brain function is not fully understood. Here we show that oxidation inhibits signaling pathways essential for synaptic plasticities in the cerebellum. We first revealed that nitric oxide (NO)-dependent plasticities at parallel fiber-Purkinje cell synapse (PF synapse) were impaired in the cerebellar slices from aged mice, suggesting possible inhibitory action of protein oxidation by endogenous reactive oxygen species. PF-synaptic plasticities were also blocked in the cerebellar slices from young mice preincubated with oxidizing agents or thiol blocker. Because the treatment of the slices with the oxidizing agent did not affect basic electrophysiological properties of PF-excitatory postsynaptic current (EPSC) and did not occlude the synaptic plasticities, oxidation was revealed to specifically inhibit signaling pathways essential for the PF-synaptic plasticities. Finally, biochemical analysis confirmed the idea that inhibitory action of protein oxidation on the PF-synaptic plasticities was mediated by impairment of NO-induced protein S-nitrosylation. Therefore, oxidation was revealed to inhibit S-nitrosylation dependent signaling pathway essential for synaptic plasticity in a ''competitive'' manner.

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Quantal Nature of Excitatory Synaptic Transmission in Cultured Hippocampal Cells of Rat and AMPAR Channel Kinetics
Masanori Nikaidoh, Akihito Kawaguchi, Yoshiyuki Saitoh, Shiori Katsumata, Hiroshi Kojima.
Fast excitatory postsynaptic currents (EPSCS) mediated by glutamate arise from AMAPR channels in the membrane of cultured hippocampal cells in culture, incubated for 2-3 weeks after the cultural procedure of dissected cells from embryonic rat brain (E 17), were identified under Nomarski optics and investigated electrophysiologically. Whole-cell current recording using the patch-clamp technique revealed the synaptic currents ranging from less than 10 to more than 200 pA at a holding potential of −80 mV. These currents were blocked by 4μM CNQX, indicating that they results from the activation of postsynaptic AMPA receptor channels. Addition of tetrodotoxin (TTX, 1μM) resulted in the loss of most currents of more than 50 pA in amplitude. The currents, which disappeared after these treatments, were seemed to be the results of spontaneous presynaptic action potentials. Moreover, 2μM-bicuculline was added in order to eliminate the inhibitory postsynaptic currents (IPSC) that were mediated by $GABA_A$ receptor channels. The peak of spontaneous miniature EPSC amplitude histograms was asymmetrical and a tail of larger amplitude miniature EPSCs was observed. The decay time courses of miniature EPSCs were fitted with single exponential component. The histogram of the decay time constants also conformed to asymmetrical distribution with a tail of longer time constant.
We also recorded the currents responses activated by (IR and/or UV) laser photolysis which have been already shown previously. It was shown that the miniature EPSCs and laser-evoked current responses have the similar physiological properties, which assumed that these currents are mediated by a AMPA receptor channel kinetics model that has been already presented.

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A mutation in the Muscle Nicotinic Receptor Alpha Subunit Leads to Slow Channel Syndrome Through Interaction with Gamma and Not the Epsilon Subunit
Michael Walogorsky, Rebecca Mongeon, Paul Brehm.
Slow channel syndrome (SCS), a congenital form of myasthenia, results from a gain of function mutation in the muscle nicotinic acetylcholine receptor (AChR). The mutant line *twister* represents a zebrafish equivalent of SCS as a result of a L258P mutation in the M2 region of the α-subunit. Zebrafish heterozygous for the *twister* mutation lack the ability to perform coordinated swimming functions during early development, presumably due to the greatly prolonged end-plate currents (EPC) resulting from altered AChR kinetics. EPCs recorded from fast skeletal muscle of *twister* decay along a triple exponential time course (1.1, 8.6 and 70.2 ms) compared to a single exponential decay typical of wild-type muscle. Paradoxically, swimming behavior improves